

## REMARKS

Reconsideration of the subject application in light of the following remarks, pursuant to and consistent with 37 C.F.R. § 1.116, is respectfully requested.

**1. Status of the Claims**

Claims 1-11 stand pending and rejected.

**2. Acknowledgement of Priority**

Applicants appreciate the Office's indication that copies of the certified copies of the priority documents have been received in the instant application from the International Bureau.

**3. Information Disclosure Statement**

Applicants appreciate the Office's acknowledgement of the Information Disclosure Statement submitted April 13, 2009.

**4. Acknowledgement of Withdrawn Objections and Rejections**

Applicants acknowledge with appreciate that the Office withdraws the following objection and rejections:

- 1) the objection of claim 1;
- 2) the rejection of claims 1-8 under 35 U.S.C. § 112, second paragraph (indefiniteness);  
and
- 3) the rejection of claim 8 under 35 U.S.C. § 112, second paragraph (indefiniteness).

Office Action, page 2, ¶ 1.

**5. Rejection of the Claims Under 35 U.S.C. § 103(a)**

**5.1. Rejection of Claims 1 and 3-11**

The Office rejects claims 1 and 3-11 under 35 U.S.C. § 103(a) as allegedly unpatentable over **JP 3163127** ("the '127 patent) in view of **U.S. Patent No. 3,882,635** ("Yamanaka").

In the Amendment / Response filed April 13, 2009, Applicants traverse Office's rejection for the following reasons:

- 1) the cited art fails to teach either varying the AN/TN ratio or the claimed AN/TN range;
- 2) the '127 patent actually teaches away from lowering AN/TN ratio; and
- 3) the successful cultivation of the algae in a medium with a low AN/TN ratio amounts an unexpected result.

However, the Office discounted the above arguments, alleging:

- 1) "the variation of organic and amino nitrogen sources are [*sic*] in fact taught by the references";
- 2) the '127 patent teaches that amino acids "may be preferential under certain conditions," which is *not* teaching away; and
- 3) the successful culture with a low AN/TN medium is *not* unexpected, because a skilled artisan "would have looked to alternate nitrogen sources" to lower production costs, regardless of disadvantages.

Office Action, pages 7-9, ¶ 17-19.

Applicants traverse the rejection. A finding of obviousness under 35 U.S.C. § 103 requires that (1) both the suggestion of the claimed invention, and (2) the expectation of success must be in the prior art, not in the disclosure of the claimed invention. *In re Dow Chem. Co.*, 837 F.2d 469, 5 U.S.P.Q.2d 1529 (Fed. Cir. 1988). Additionally, (3) "obviousness requires a suggestion of *all* limitations in a claim." *CFMT, Inc. v. Yieldup Int'l Corp.*, 349 F.3d 1333, 1342, 68 U.S.P.Q.2d 1940, 1947 (Fed. Cir. 2003) (citing *In re Royka*, 490 F.2d 981, 985, 180 U.S.P.Q. 580, 583 (C.C.P.A. 1974) (emphasis added)). Furthermore, one common inquiry in the above tests of obviousness is whether an ordinarily skilled in the art would have reasonable expectation of success to practice the claimed invention. *Examination Guidelines for Determining Obviousness under 35 U.S.C. 103 in View of the Supreme Court Decision in KSR International Co. v. Teleflex Inc.*, 72 Fed. Reg. 57,528.

The Office's assertions are unsupported. First, the cited art provides neither suggestion of the claimed method nor expectation of success to practice the claimed invention. The '127 patent and Yamanaka, alone or when viewed in combination, fail to suggest at least that a medium with the recited amount of organic nitrogen (reflected by the AN/TN ratio) could be used to cultivate green algae to produce astaxanthin (claim 1). The '127 patent is the primary reference. The '127 patent teaches no more than a medium. The '127 patent does not correlate

astaxanthin production with the culture medium. Specifically, the '127 patent teaches culturing *Haematococcus pluvialis* NIES 144 in a medium with the following composition: yeast extract (2.0 g/L); Na-acetate (1.2 g/L); L-asparagine (0.4 g/L);  $\text{MgCl}_2 \cdot 7\text{H}_2\text{O}$  (984  $\mu\text{M}$ );  $\text{FeSO}_4 \cdot 7\text{H}_2\text{O}$  (36  $\mu\text{M}$ ); and  $\text{CaCl}_2 \cdot 2\text{H}_2\text{O}$  (136  $\mu\text{M}$ , pH 6.8). There is no specific aspect of the medium that is indicated to be more important than the rest.

The Office relies on Yamanaka, the secondary reference, for the assertion that a skilled artisan would have been motivated to change the nitrogen source for cultivating algae. However, the assertion relying on Yamanaka is far-fetched. Yamanaka describes:

A combination of carbon and nitrogen sources suitable for sustaining the growth of *P. sphaerica* occurs in the **waste waters** of many industries processing natural organic substances, particularly the food industry. The alga has been grown successfully on **waste water** from sugar factories, breweries, and other fermentation plants producing amino acids, nucleic acids, and antibiotics, meat processing plants, dairies, fish canneries, bakeries, flour mills, and tanneries.

Yamanaka, col. 2, line 61 to col. 3, line 2 (emphasis added).<sup>1</sup> At best, this section teaches that *P. sphaerica* may be able to utilize carbon and nitrogen sources **in various waste materials**. The exact nitrogen sources present in the waste materials and their quantity are undisclosed. Additionally, the nitrogen sources are in **waste** materials, which also contain numerous other materials present. The references fail to suggest which of the many available nitrogen sources could be successfully used as presently claimed. The skilled artisan would have had to identify which nitrogen materials were present, their quantity, and then test them.

Given Yamanaka fails to provide what nitrogen materials are present and their concentration, no AN/TN ratios can be determined. Accordingly, the Office cannot conclude that it reads upon the claimed AN/TN ratio. Without all claims elements being taught, the cited reference could not have provided a skilled artisan with a reasonable expectation of success in practicing the claimed method, thus failing this element of the obviousness test.

Second, the Office mischaracterizes the teaching away of the '127 patent as to the recited AN/TN ratio. The Office is reminded that a claim is interpreted by "taking into account

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<sup>1</sup> The Office refers to col. 2, lines 62-68 of Yamanaka. Office Action, page 8. For the clarity of the record, Applicants cite herewith the full paragraph containing the cited portion.

whatever enlightenment by way of definitions or otherwise that may be afforded by the written description contained in applicant's specification." See *In re Morris*, 127 F.3d 1048, 1054-55, 44 U.S.P.Q.2d 1023, 1027-28 (Fed. Cir. 1997). As defined in the Specification, the claimed AN/TN ratio refers to the proportion of amino nitrogen to the total nitrogen. See *e.g.*, Specification, page 6, ¶ [0009]; page 9, ¶ [0013].<sup>2</sup> For example, the higher the degree of hydrolysis of a protein preparation, the higher the AN/TN ratio. See *id.*, page 9, ¶ [0013]; see *e.g.*, U.S. Patent No. 6,692,933, col. 3, lines 45-53 (attached as Appendix I).<sup>3</sup>

Similarly, a high AN/TN ratio corresponds a high level of free amino nitrogen. A medium having amino acids, urea, or nitrate as the nitrogen source would have a high AN/TN ratio. See *e.g.*, Specification, pages 9-10, ¶ [0015]. Conversely, a low AN/TN ratio corresponds a low level of free amino nitrogen. For example, a medium having a high level of corn steep liquor, soy bean powder, peptone, tripeptone, or polypeptone (as recited in claim 3) would have a low AN/TN ratio. See *id.* The presently claimed methods recite culturing green algae in a medium having a **low** AN/TN ratio. Thus, any reference that teaches or suggests a medium with a **high** level of free amino nitrogen (*i.e.*, a high AN/TN ratio or using amino acids, urea, and/or nitrate as the nitrogen source) teaches away the claimed methods.

The two paragraphs of the '127 patent's English translation indicates that amino acids can substitute for yeast extract as the nitrogen source. See pages 6-7, Amendment / Response filed April 13, 2009. The media with amino acids as the nitrogen source would have a high AN/TN ratio. See *supra*.

The Office is further directed to the paragraph bridging pages 4-5 of the Specification (¶ [0006]):

<sup>2</sup> "According to the present invention, an organic nitrogen source having a low proportion of amino nitrogen to the total nitrogen (AN/TN ratio) is used as an organic nitrogen source to be added to the basal medium. The amino nitrogen as used herein refers to all forms of nitrogen other than that in the amino acid residues in peptides and it chiefly means the nitrogen in free amino acids."

<sup>3</sup> "A measure for the degree of hydrolysis of protein is the AN/TN ratio. AN is the amino-nitrogen level, which can be determined using the formol titration method, or according to J. Adler-Nissen, Enzymatic hydrolysis of food proteins. Elsevier Applied Science Publishers, 1986. TN is the total amino-nitrogen content which is determined according to the Kjeldahl nitrogen determination method. The higher the ratio AN/TN, the higher the degree of hydrolysis of the protein preparation."

[0006] In the cultivation of *Haematococcus pluvialis* which is performed to produce astaxanthin, ***inorganic nitrogen is primarily used as the required nitrogen source in the medium for cell construction*** and exemplary nitrogen sources that have been used in the medium include nitrates (Appln. Microbiol. Biotechnol. 53:530-535 (2000)) and urea (Appln. Microbiol. Biotechnol. 53:537-540 (2001)). On the other hand, ***organic nitrogen sources such as high-protein nitrogen sources including defatted soya bean powder have an extremely high tendency to foam*** and contain large amounts of water-insolubles, so ***uniform mixing is difficult to achieve in a large, outdoor, open reactor***; CSL (corn steep liquor) which is the liquid by-product of sugar purification does not have uniform quality, especially in terms of the quantity of the microorganism that enters and ***this is likely to induce microbial contamination in the outdoor, open reactor for which it is difficult in to sterilize the medium***. In addition, in order to minimize the entrance and growth of heterotrophic microorganisms which are the primary cause of microbial contamination and to ensure that the growth of those green algae which are capable of autotrophic growth by photosynthesis will predominate, it has been attempted to keep the medium ingredients in a state that is as oligotrophic as possible. ***For these reasons, only a few cases have been reported of using the organic nitrogen source in astaxanthin production by cultivation of green algae.***

(emphasis added). The above paragraph indicates that nitrogen sources such as nitrates and urea are primarily used to cultivate green algae at the time of the invention. Media with nitrates or urea as the nitrogen source have a high AN/TN ratio. *See supra*. Additionally, the organic nitrogen sources such as defatted soy bean powder and corn steep liquor are considered unsuitable for cultivating green algae because of the above-discussed disadvantages. Media with soy bean powder or corn steep liquor as the nitrogen source would have a low AN/TN ratio. *See supra*. Viewing the teaching of the '127 patent and the above description in combination, ***a skilled artisan would have regarded a medium with a low AN/TN ratio undesirable for cultivating green algae.*** Accordingly, the '127 patent in fact teaches away from the presently claimed methods. The Office is respectfully requested to reconsider the '127 patent's teachings in view of the instant application.

Third, the Office overlooks the disadvantages of using a medium having organic nitrogen sources such as defatted soy bean powder and corn steep liquor—having a low AN/TN ratio. The Office asserts that a skilled artisan “would therefore have looked to alternate nitrogen sources in such a process, ***regardless*** of foaming, because would have lowered production costs.”

Office Action, page 9 (emphasis added). The Office is reminded that a skilled artisan “is a person of ordinary creativity, not an automaton.” *See KSR Int’l Co. v. Teleflex Inc.*, 550 U.S. 398, 421, 82 U.S.P.Q.2d 1385, 1397 (2007). Even if it were assumed *arguendo* that alternate nitrogen sources (*e.g.*, defatted soy bean powder and corn steep liquor) are able to lower production costs, a skilled artisan would not have ignored their known disadvantages.<sup>4</sup> The Office fails to provide any evidence or justification that the reduced production costs would have outweighed the known disadvantages, let alone that overcoming these disadvantages could have been foreseeable and had any reasonable expectation of success. The only way the Office appears to achieve combining the elements from the references is through the hindsight roadmap of Applicants’ own specification. The Office is not permitted to pick and choose between all the options and teachings presented by each of the references to arrive at the combination of limitations as presented in the claims. *See AKZO N.V. v. United States Int’l Trade Comm’n*, 808 F.2d 1471, 1781, 1 U.S.P.Q.2d 1241, 1246 (Fed. Cir. 1986) (one “cannot pick and choose among individual parts of assorted prior art references as a mosaic to recreate a facsimile of the claimed invention.”).

In view of the above remarks, the Office’s rejection of claims 1 and 3-11 is unsupported. Applicants respectfully request withdrawal of the obviousness rejection and allowance of the claims.

## **5.2. Rejection of Claims 1-11**

The Office rejects claims 1-11 under 35 U.S.C. § 103(a) as allegedly unpatentable over the ‘127 patent in view of Yamanaka as applied to claims 1 and 3-11, and further in view of **Tanaka** (WO 02/077105) (“Tanaka”). The Office relies upon Tanaka for its purported teaching of a method of extracting an astaxanthin-containing lipid from ruptured algae. Office Action, pages 8-9, ¶ 18.

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<sup>4</sup> The Office apparently regards the “waste materials” of Yamanaka as “alternate nitrogen sources” having “lowered production costs.” However, the exact components in the waste materials are unknown. *See* section 5.1 *supra*. Without any justification, the Office further assumes that the “waste materials” of Yamanaka are suitable for culturing the algae to produce astaxanthin.

In the Amendment / Response filed April 13, 2009, Applicants points out that Tanaka fails to cure the defects of the '127 patent and Yamanaka, because Tanaka at least does not teach the claimed AN/TN ratio. The Office admits that Tanaka fails to suggest the claimed AN/TN ratio. Office Action, page 8, ¶ 18. The Office, however, asserts that the teaching of the claimed AN/TN ratio is provided in the other references, presumably the '127 patent and Yamanaka. *Id.*, at 8-9, ¶ 18.

Applicants traverse. The '127 patent and Yamanaka, alone or when viewed in combination, fail to (1) teach the claimed AN/TN ratio, and (2) suggest lowering the AN/TN ratio of a medium for astaxanthin production as argued in Section 5.1 *supra*. Tanaka fails to cure the defects, because Tanaka is relied upon for allegedly teaching an extraction method. Tanaka does not teach the claimed AN/TN ratio. Nor does Tanaka suggest cultivating green algae in a medium with a low AN/TN ratio. The references, alone or when view in combination, fail to teach all claim elements. Thus, a skilled artisan would have had no reasonable expectation to practice the claimed method. Accordingly, the Office's rejection of claims 1-11 is unsupported. Applicants request withdrawal of the obviousness rejection and allowance of the claims.

### **CONCLUSION**

Should the Examiner have any questions or comments regarding Applicants' amendments or response, please contact Applicants' undersigned representative at (202) 842-8821. Furthermore, please direct all correspondence to the below-listed address.

In the event that the Office believes that there are fees outstanding in the above-referenced matter and for purposes of maintaining pendency of the application, the Office is authorized to charge the outstanding fees to Deposit Account No. 50-0573. The Office is likewise authorized to credit any overpayment to the same Deposit Account Number. If an Appeal fee is required to maintain pendency of the present application, the Office is authorized to charge the Appeal fee to the deposit account above and use this paper as a Notice of Appeal.

Respectfully submitted,

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# Appendix I

(12) **United States Patent**  
Merrill et al.

(10) Patent No.: **US 6,692,933 B2**  
(45) Date of Patent: **Feb. 17, 2004**

(54) **METHOD FOR PRODUCING A  
GLUTEN-FREE PEPTIDE PREPARATION  
AND PREPARATION THUS OBTAINED**

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(\*) Notice: Subject to any disclaimer, the term of this  
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U.S.C. 154(b) by 0 days.

(21) Appl. No.: **10/043,660**

(22) Filed: **Jan. 9, 2002**

(65) **Prior Publication Data**

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**Related U.S. Application Data**

(60) Provisional application No. 60/261,631, filed on Jan. 12,  
2001.

(51) Int. Cl.<sup>7</sup> ..... **C12P 21/06**

(52) U.S. Cl. .... **435/68.1**

(58) Field of Search ..... **435/68.1**

(56) **References Cited**

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Tanabe et al., "Production of a High-Glutamine Oligopeptide Fraction from Gluten by Enzymatic Treatment and Evaluation of its Nutritional Effect on the Small Intestine of Rats," *Journal of Food Biochemistry*, 1993, pp. 235-248, vol. 16.

AOAC, "Gluten in Foods: Colorimetric Monoclonal Antibody Enzyme Immunoassay Method," *Official Methods of Analysis (1990) 15<sup>th</sup> Edition: 2<sup>nd</sup> Supplement*, 1991, pp. 94-96.

Wilcox, "Determination of Amide Residues by Chemical Methods," *Methods of Enzymology*, 1967, pp. 63-76, vol. 11.

Adler-Nissen, *Enzymic Hydrolysis of Food Proteins*, 1986, pp. 12-13, 122-123, Galliard (Printers) Ltd, Great Yarmouth, Great Britain.

Friedli, "Interaction of Deamidated Soluble Wheat Protein (SWP) with Other Food Proteins and Metals: A Thesis presented for the Award of Doctor of Philosophy to the University of Surrey," 1996.

\* cited by examiner

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(57) **ABSTRACT**

The invention relates to a method for producing a glutamine-rich gluten-free peptide preparation from gluten protein, comprising the steps of enzymatically hydrolysing wheat gluten using one or more proteases to obtain a hydrolysate; acidifying the hydrolysate to a pH between 4 and 5; and filtering the hydrolysate to obtain the glutamine-rich gluten-free peptide preparation as the filtrate. The protease is preferably a neutral or basic protease and the optimum pH is from, 4.5 to 4.7.

**16 Claims, No Drawings**

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# **METHOD FOR PRODUCING A GLUTEN-FREE PEPTIDE PREPARATION AND PREPARATION THUS OBTAINED**

## **CROSS-REFERENCE TO RELATED APPLICATIONS**

This application claims the benefit of U.S. Provisional Application No. 60/261,631, filed Jan. 12, 2001 entitled "Gluten-Free Peptide Preparation" and European Application No. 01200387.7, filed Feb. 2, 2001, entitled "Method For Producing A Gluten-Free Peptide Preparation And Preparation Thus Obtained".

## **BACKGROUND OF THE INVENTION**

The present invention relates to a method for producing a peptide preparation that is both glutamine-rich and gluten-free and to the preparation thus obtained. The invention also relates to the use of the preparation in various products and to the products containing the preparation.

Gluten is a combination of proteins found in the endosperm of various grains, such as wheat, barley and rye, oats and other gluten-containing wheat variants, such as triticale, spelt and kamut. In wheat, gluten accounts for 90% of the protein and makes up almost 15% of the total weight of a grain. It is thus an important source of protein.

However, gluten is the cause of a genetic disorder known as coeliac disease or gluten intolerance. Symptoms of coeliac disease can range from the classic features, such as diarrhea, weight loss, and malnutrition, to latent symptoms such as isolated nutrient deficiencies. The disease mostly affects people of European descent, and occurs more rarely in black and Asian populations. Those affected suffer damage to the villi (shortening and villous flattening) in the lamina propria and crypt regions of their intestines when they eat specific food-grain antigens (toxic amino acid sequences) that are found in wheat, rye, and barley, oats and other gluten-containing wheat variants, such as triticale, spelt and kamut. The gluten found in rice and corn do not cause the intolerance.

For persons with coeliac disease the toxic part of the gluten molecule is the prolamins portion: gliadin in wheat, secalin in rye and horedin in barley. Following a gluten-free diet, people can recover from the symptoms of the disease, but they cannot be cured. Re-introduction of gluten in the diet will again lead to symptoms.

Glutamine is an amino acid that occurs abundantly in gluten. Although it is not an essential amino acid it is nevertheless desirable for certain individuals, in particular those who are recovering from surgery, suffering from gastrointestinal disorders, immune function deficiencies, metabolic stress states, shock or performing endurance sports. Such individuals would benefit from supplementation with this amino acid, for example by taking a peptide preparation rich in glutamine.

Gluten is a very cost-effective source for such glutamine-rich peptide preparations. However, the known preparations are not suitable for coeliac patients since they still contain the toxic parts of the gliadin.

It is therefore the object of the present invention to provide a peptide preparation that is rich in bound glutamine but at the same time gluten free.

## **SUMMARY OF THE INVENTION**

Such peptide preparation can be obtained by a method, comprising the steps of:

- a) enzymatically hydrolysing gluten using one or more proteases to obtain a hydrolysate;

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- b) acidifying the hydrolysate to a pH between 4 and 5; and
- c) filtering the hydrolysate to obtain the glutamine-rich gluten-free peptide preparation as the filtrate.

## **DETAILED DESCRIPTION OF THE INVENTION**

A glutamine-rich gluten-free is obtained by a method, comprising the steps of:

- a) enzymatically hydrolysing gluten using one or more proteases to obtain a hydrolysate;
- b) acidifying the hydrolysate to a pH between 4 and 5; and
- c) filtering the hydrolysate to obtain the glutamine-rich gluten-free peptide preparation as the filtrate.

The term "gluten-free" is intended to indicate that the product when tested in an ELISA based on anti- $\Omega$ -gliadin antibodies yields a value of <200 ppm. A suitable ELISA to test the gluten-free property is as described in the Association of Official Analytical Chemists' (AOAC's) Official Methods of Analysis, 15th Edition, 2nd supplement (1991).

It is clear that the proteases to be used can be selected from a wide range of proteases known in the art provided that hydrolysis performed with such protease results in a preparation that yields <200 ppm in the above described ELISA. Proteases include acid, basic and neutral proteases derived from bacterial, fungal, animal or botanical sources. It was found that basic or neutral proteases active at a pH above 6 are particularly well suited. Examples of such proteases are Proleather N (Amano), Neutrase (NOVO), PROMOD 192P (Biocatalysts), Alcalase 2.4L (NOVO), Protease S (Amano), Peptidase A (Amano), Peptidase R (Amano). Of these the following proteases are preferred: Proleather N (Amano) and Alcalase 2.4L (NOVO).

The protein fragments that cause the hypersensitivity in coeliac patients are surprisingly removed when the hydrolysate is acidified and subsequently filtered. It is assumed that these fragments are precipitated and remain in the retentate of the filter. The pH to which the hydrolysate is to be acidified lies between 4 and 5, preferably between 4.1 and 4.9, more preferably between 4.3 and 4.8, most preferably between 4.5 and 4.7, and is optimally 4.6.

Hydrolysis is an essential step in the method of the invention as without hydrolysis the toxic fragments cannot be removed.

Peptide preparations that are obtainable by the method of the invention consisting of peptides that do not induce gluten hypersensitivity symptoms in coeliac patients are a further aspect of this invention. Such preparations are suitable as a food additive or food stuff for supplying additional glutamine to a subject. The preparation thus has sports and clinical applications and can be used in enteral nutrition and pet food.

The peptide preparation of the invention can be used in further products that can be taken by or administered to subjects in need of supplementation. Particular embodiments of such products are glutamine peptide tablets comprising the usual carriers, diluents and excipients for tablets and a peptide preparation of the invention as glutamine peptide source, glutamine peptide liquid beverage comprising the usual ingredients for beverages and a peptide preparation of the invention as glutamine peptide source, and glutamine peptide enteral nutrition comprising the usual carriers, diluents and excipients for enteral nutrition and a peptide preparation of the invention as glutamine peptide source.

Although the invention is more broadly applicable to gluten from all grains that may cause coeliac disease, it is preferred to use wheat because of its high glutamine content.

The present invention will be further elucidated in the following examples that are given for illustration purposes only and are in no way intended to limit the scope of the invention.

### EXAMPLES

#### Example 1

##### Production of a Glutamine-Rich, Gluten-Free Peptide Preparation

A series of experiments was carried out to illustrate the critical process parameters.

A series of peptide hydrolysates was produced by heating deionized water to a temperature of  $63^{\circ}\text{C} \pm 1^{\circ}\text{C}$ . To this water, a mix of 45% liquid potassium hydroxide, 50% liquid sodium hydroxide, hydrated calcium hydroxide in a ratio of 1:0.78:0.70, respectively, is added to obtain a pH suitable for the protease to be used.

Vital wheat gluten ("VWG", Cargill B. V., Bergen op Zoom, Netherlands) is added to this solution to produce a 12% solids mix of solubilized gluten.

Hydrolysis is performed with a desired protease as indicated in the description of the separate experiments hereinbelow. The hydrolysis reaction is performed for 3 hours at a temperature that is suitable for the protease used, usually  $60^{\circ}\text{C} \pm 2^{\circ}\text{C}$ .

After the hydrolysis, acid, in particular sulphuric acid is added to achieve the desired pH (see description of experiments) with agitation. The reaction is stopped by a HTST (high temperature short time) heating at  $116^{\circ}\text{C} \pm 2^{\circ}\text{C}$ . Subsequently the solution is cooled to  $66^{\circ}\text{C} \pm 2^{\circ}\text{C}$  and filtered using diatomaceous earth (Eagle-Picher Minerals Inc., Reno, Nev., USA) at 40% bodyfeed. The solution is recirculated through the filter press for a minimum of 3 minutes.

The pH of the filtrate is adjusted to 6.4-6.8 by means of an alkaline solution. After evaporating the liquid and drying, a powdered peptide preparation of the invention is obtained.

In order to test whether the product is gluten-free an ELISA was performed according to AOAC 991.19 (Official Methods of Analysis (1990) 15th Edition, 2nd a Supplement (1991)).

The bound glutamine content was determined according to P. E. Wilcox, "Determination of Amide Residues by Chemical Methods." Methods of Enzymology 11, 63-76 (1967).

A measure for the degree of hydrolysis of protein is the AN/TN ratio. AN is the amino-nitrogen level, which can be determined using the formol titration method, or according to J. Adler-Nissen, Enzymatic hydrolysis of food proteins. Elsevier Applied Science Publishers, 1986. TN is the total amino-nitrogen content which is determined according to the Kjeldahl nitrogen determination method. The higher the ratio AN/TN, the higher the degree of hydrolysis of the protein preparation.

#### Experiments

##### Experiment 1

Wheat gluten is dispersed in water. The pH is adjusted to 4.6 with sulphuric acid and the solution filtered.

##### Experiment 2

Wheat gluten is dispersed in water. The pH is adjusted to 3.2-3.4 with sulphuric acid. The gluten is digested using Acid Protease II (Amano). The enzyme is heat inactivated and the solution is filtered.

##### Experiment 3

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase

2.4L (NOVO) and Proleather N (Amano), and amylases BAN 240L (NOVO). After inactivation of the enzyme the pH is adjusted to near neutral with sulphuric acid and the solution is filtered.

##### Experiment 4

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase 2.4L (NOVO) and Proleather N (Amano). The pH is adjusted to 3.8-4.1 with sulphuric acid. After heat inactivation of the enzyme the solution is filtered. The pH is then adjusted to neutral with caustic.

##### Experiment 5

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase 2.4L (NOVO) and Proleather N (Amano). The pH adjusted to 6.5 with sulphuric acid. After heat inactivation of enzymes, the solution is filtered. The pH is adjusted to neutral using caustic.

##### Experiment 6

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase 2.4L (NOVO) and Proleather N (Amano). The pH is adjusted to 4.3 with sulphuric acid. After heat inactivation of enzymes and filtration, the pH is adjusted to neutral using caustic.

##### Experiment 7

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase 2.4L (NOVO) and Proleather N (Amano). Subsequently the pH is adjusted to 4.5 with sulphuric acid. After heat inactivation of enzymes and filtration, the pH is adjusted back to neutral using caustic.

##### Experiment 8

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase 2.4L (NOVO) and Proleather N (Amano). The pH is adjusted to 4.6 with sulphuric acid. After heat inactivation of enzymes the solution is filtered. Then the pH is adjusted to neutral using caustic.

##### Experiment 9

Wheat gluten is dispersed in caustic water. The gluten is digested using the alkaline and neutral proteases Alcalase 2.4L (NOVO) and Proleather N (Amano). The pH is adjusted to 4.8 with sulphuric acid. After heat inactivation of enzymes and filtration, the pH is adjusted to neutral using caustic.

Table 1 shows the result of the experiments. It is clear from the above example that both hydrolysis of the gluten and filtration at an acid pH are essential for the product to be gluten free.

TABLE 1

Sample	AN %	Gluten (ppm)	Bound Glutamine (%)
1	0.5	1200	19
2	0.67	>320	31
3	1.4	438	25
4	1.62	300	25

TABLE 1-continued

Sample	AN %	Gluten (ppm)	Bound Glutamine (%)
5	1.59	310	27
6	1.7	<20	28
7	2.03	<20	27
8	1.95	<20	26
9	1.96	<20	27

TN = 13%

## Example 2

## Application of Gluten-Free Glutamine-Rich Peptide Preparation of the Invention

In the following, three examples of applications for the preparation of the invention are given.

## 1. Glutamine Peptide Tablets

## Ingredients

- (1) Enzymatically Hydrolysed Wheat Protein (granular) (preparation according to the invention)
- (2) Pharmcel 102
- (3) CAB-0-SIL M-5

## Recipe:

Enzymatically hydrolyzed wheat protein (1)	91.1%
Microcrystalline cellulose (2)	5.0%
Di-calcium phosphate	2.0%
Silicon Dioxide (3)	0.9%
Stearic Acid	0.5%
Magnesium Stearate	0.5%
Total	100%

## Preparation Method

The powders are premixed (withholding the Mg Stearate until the last minutes of mixing). The tablets are prepared by direct compression.

## Properties of the tablets:

Glutamine Peptide per tablet	170 mg
Tablet weight	758 mg
Tablet length (Oblong)	19.04 mm
Compression pressure	13.3 kN
Hardness	140 N

## 2. Glutamine Peptide Liquid Beverage

## Ingredients

- (1) Enzymatically hydrolyzed wheat protein (preparation of the invention)
- (2) Enzymatically hydrolyzed whey protein (WE80BG, DMY International)
- (3) Grapefruit Flavor Tastemaker 946068

## Recipe:

Water (QS to 1 liter)	920.00 g
Enzymatically Hydrolyzed Wheat Gluten (1)	13.21 g
Enzymatically Hydrolyzed Whey (2)	13.04 g
Sucrose	26.60 g
Glucose	15.00 g

## -continued

## Recipe:

5	Fructose	5.00 g
	Glucose Polymers (Maltodextrin DE18)	10.00 g
	Malic Acid	3.33 g
	Citric Acid	0.67 g
	Sodium Citrate	1.00 g
	Grapefruit Flavor (3)	0.60 g
10	Aspartame	0.10 g
	Acesulfame Potassium	0.10 g
		1000.0 ml

## Preparation method

All ingredients are added to the water and mixed well. The acids are added last to achieve a pH of 3.9. The liquid is bottled, heat processed for 1 min. at 85° C. and cooled,

## Nutrition Facts (per 100 ml):

25	Protein	2.09 g
	Glutamine Peptide	0.26 g
	Carbohydrates	6.0 g

## 3. Clinical Enteral Nutrition Prototype with Glutamine Peptide and Whey Peptides

## Ingredients

- (1) Enzymatically hydrolyzed wheat protein (preparation of the invention)
- (2) Enzymatically hydrolyzed whey protein (WE80BG, DMV International)

## Recipe:

40	Water (QS to 1 liter)	720.00 g
	Enzymatically Hydrolyzed Wheat Gluten (1)	40.00 g
	Enzymatically Hydrolyzed Whey (2)	35.60 g
	Food Starch, Modified	84.00 g
	Maltodextrin	59.00 g
45	Soy Oil	30.00 g
	MCT Oil	10.00 g
	Potassium Citrate	2.20 g
	Sodium Citrate	1.60 g
	Magnesium Chloride	3.20 g
	Calcium Phosphate	2.80 g
50	Potassium Phosphate	2.00 g
	Sodium Phosphate	1.00 g
	Carrageenan	0.50 g
		1000.0 ml

## Preparation Method

The minerals are dissolved in water with constant stirring. The premixed carbohydrates are added to the mixture. The mixture is heated to 70° C. and held for 10 minutes with constant stirring. The protein is added to the mixture, which is then heated to 70° C. with constant stirring. The oil is added to the mixture, which is then mixed well. The mixture is then double homogenised at 4000 psig (276 bar). The pH is adjusted to the appropriate value. The solids content is adjusted to an appropriate value. The product is sterilised and the heat process retorted at 121° C. for 10 minutes.

Nutrition Facts (per 100 ml):	
Protein	6.0 g
Glutamine Peptide	1.0 g
Carbohydrates	13.8 g
Fat	4.0 g

What is claimed is:

1. A method for producing a glutamine-rich gluten-free peptide preparation from gluten protein, comprising the steps of:

- a) enzymatically hydrolysing gluten using one or more proteases to obtain a hydrolysate;
- b) acidifying the hydrolysate to a pH between 4 and 5; and
- c) filtering the hydrolysate to obtain the glutamine-rich gluten-free peptide preparation as the filtrate.

2. The method as claimed in claim 1, wherein the proteases are alkaline or neutral proteases.

3. The method as claimed in claim 1, wherein the pH is between 4.2 and 4.8.

4. The method as claimed in claim 1, wherein the pH is between 4.5 and 4.7.

5. The method as claimed in claim 1, wherein proteases are used that are active at a pH above 6.

6. The method as claimed in claim 1, wherein between step b) and c) the enzymes are inactivated.

7. The method as claimed in claim 6, wherein the enzymes are inactivated by means of heat.

8. The method as claimed in claim 1, wherein the gluten is wheat gluten.

9. A preparation prepared from gluten protein, which is glutamine-rich and gluten-free and is obtained by the method of claim 1.

10. The peptide preparation as claimed in claim 9, wherein the gluten from which the preparation is made is wheat gluten.

11. The peptide preparation as claimed in claim 9 for use as an ingredient in glutamine peptide tablets.

12. The peptide preparation as claimed in claim 9 for use as an ingredient in glutamine peptide liquid beverages.

13. The peptide preparation as claimed in claim 9 for use as an ingredient in glutamine peptide enteral nutrition.

14. Glutamine peptide tablets comprising the carriers, diluents and excipients for tablets and a peptide preparation as claimed in claim 9 as glutamine peptide source.

15. A glutamine peptide liquid beverage comprising ingredients for beverages and a peptide preparation as claimed in claim 1 as glutamine peptide source.

16. A glutamine peptide enteral nutrition comprising carriers, diluents and excipients for enteral nutrition and a peptide preparation as claimed in claim 9 as glutamine peptide source.

\* \* \* \* \*

UNITED STATES PATENT AND TRADEMARK OFFICE  
**CERTIFICATE OF CORRECTION**

PATENT NO. : 6,692,933 B2  
DATED : February 17, 2004  
INVENTOR(S) : Debra Ann Merrill et al.

Page 1 of 1

It is certified that error appears in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

Column 2,

Line 7, "gluten-free is" should read -- gluten-free peptide is --.

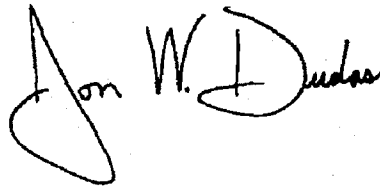
Column 8,

Line 5, "A preparation" should read -- A peptide preparation --.

Line 23, "in claim 1" should read -- in claim 9 --.

Signed and Sealed this

Twentieth Day of July, 2004

A handwritten signature in black ink, reading "Jon W. Dudas". The signature is stylized, with a large, looped initial "J" and a cursive "Dudas".

JON W. DUDAS  
*Acting Director of the United States Patent and Trademark Office*